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Hypoglycemic effect of cymbopogon citratus leaves' extract and it's fractions in alloxan-induced diabetic Mice

Temu, Joyce

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**HYPOGLYCEMIC EFFECT OF *Cymbopogon citratus* LEAVES'
EXTRACT AND IT'S FRACTIONS IN ALLOXAN-INDUCED
DIABETIC MICE**

Joyce Eliaoni Temu

**A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of
Master's in Life Sciences of the Nelson Mandela African Institution of Science and
Technology**

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ABSTRACT

Varieties of plants, including *Cymbopogon citratus*, are traditionally used in controlling hyperglycemia by either stimulating insulin secretion, inhibition α - Glucosidase or α -amylase activity. *Cymbopogon citratus* leaves were studied in this study to elucidate the effects of actively fractionated fraction from crude extract in lowering blood glucose in diabetic mice. The *C. citratus* powder were extracted by cold maceration using ethanol. Fractionation was done by Vacuum Liquid Chromatography (VLC). Oral glucose tolerance test (OGTT) was performed for both crude extract and fractions. Diabetes was induced in mice by intraperitoneal injection of freshly prepared alloxan monohydrate (170 mg/kilogram/body weight) which interferes with insulin secretion. The mice were treated with ethyl acetate fraction once daily at 400 mg/kilogram body weight dose for the period of 20 days. Fasting blood glucose (FBG) and weight were then recorded from mice in days 1, 5, 10, 15 and 20. Acute toxicity done by Lorke's method. The difference between means of two population groups was considered significant at $p < 0.05$ by One-way ANOVA. Results were expressed as mean \pm SD. Significance hypoglycemic activity was shown by ethyl acetate. No mortality was observed at 5000 mg/kilogram body weight dose but sleeping and tremor were observed at a 1000 - 5000 mg/kilogram body weight. Phytochemical screening of ethyl acetate fraction showed presence of alkaloids, saponins, anthraquinone, phenol and tannins. Good hypoglycemic effect and safety results from ethyl acetate fraction suggest that *C. citratus* extracts are against insulin-dependent hyperglycemia. Isolation and testing of active ingredients from the *C. citratus* extract are thus warranted.

Keywords: *Cymbopogon citratus*, hyperglycemia, mice, oral glucose tolerance test, acute toxicity.

DECLARATION

I, Joyce Eliaoni Temu, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither submitted nor being concurrently submitted for degree award in any institution.

Joyce Eliaoni Temu

Candidate Name

Signature

Date

The above declaration is confirmed

Dr. Haikael D. Martin

Supervisor (1)

Signature

Date

Dr. Elingarami Sauli Nkya

Supervisor (2)

Signature

Date

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CERTIFICATION

This is to certify that the accompanying dissertation by Joyce Eliaoni Temu has been accepted in partial fulfillment of the requirement for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Dr. Haikael D. Martin

Supervisor (1)

Signature

Date

Dr. Elingarami Sauli Nkya

Supervisor (2)

Signature

Date

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DEDICATION

This dissertation is dedicated to my beloved son Baithin Kitali, my friend Abdulazak Mbekomize, my brother CallingGod Temu for their love and constant support when I needed them during my studies. Also, to my parents Mr & Mrs. Eliaoni Temu and relatives for being always there for me when needed them.

TABLE OF CONTENTS

ABSTRACT.....	i
DECLARATION	ii
COPYRIGHT.....	iii
CERTIFICATION	iv
ACKNOWLEDGMENTS	v
DEDICATION.....	vi
TABLE OF CONTENTS.....	vii
LIST OF TABLES.....	x
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS AND SYMBOLS	xii
CHAPTER ONE.....	1
INTRODUCTION	1
1.1 Background of the problem	1
1.2 Statement of the problem.....	3
1.3 Rationale of the study	4
1.4 Research objectives.....	4
1.4.1 General objective.....	4
1.4.2 Specific objectives.....	4
1.5 Research questions.....	4
1.6 Significance of the study.....	5
1.7 Delineation of the study	5
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 Ethnobotany in management of human diseases	6
2.2 Prevalence of diabetes.....	6

2.3	Diabetes management interventions	7
2.4	<i>Cymbopogon citratus</i> and diabetes	7
2.5	Safety and toxicity of medicinal plants.....	9
CHAPTER THREE.....		10
MATERIALS AND METHODS.....		10
3.1	Study design.....	10
3.2	Materials, Chemicals and Reagents	11
3.3	Extraction and Fractionation of <i>Cymbopogon citratus</i>	11
3.4	Extraction of <i>Cymbopogon citratus</i> water extract	12
3.5	Animal Selection and Identification	13
3.6	Oral Glucose Tolerance Test (OGTT)	14
3.7	Induction of diabetes in mice.....	15
3.8	Extract administration and hypoglycemic activity in mice.....	15
3.9	Test animal handling.....	15
3.10	Phytochemical screening of ethyl acetate fraction.....	16
3.10.1	Experimental procedures.....	16
3.11	Acute toxicity test	17
3.12	Hypoglycemic statistical data analysis	17
CHAPTER FOUR.....		18
RESULTS AND DISCUSSION		18
4.1	Extracts and fractions yields	18
4.2	Oral glucose tolerance test (OGTT).....	18
4.3	Diabetes induction test.....	21
4.4	Phytochemical screening of <i>Cymbopogon citratus</i> ethyl acetate fraction	25
4.5	Acute toxicity test	26
CHAPTER FIVE		28

CONCLUSION AND RECOMMENDATIONS	28
5.1 Conclusion	28
5.2 Recommendations.....	28
REFERENCES	29
RESEARCH OUTPUTS.....	36

LIST OF TABLES

Table 1: Fractions and their percentage yield	18
Table 2: Mean FBG (mmol/l) for OGTT	19
Table 3: Mean FBG (mmol/l) for alloxan induce diabetes mice	22
Table 4: Mean body weights of alloxan treated mice	23
Table 5: Phytochemical screening of <i>Cymbopogon citratus</i> ethylacetate fraction	25
Table 6: Mice behavioral changes.....	27

LIST OF FIGURES

Figure 1: Appearance of <i>Cymbopogon citratus</i> Nazi village in Korogwe district.....	8
Figure 2: Flow of activities (methodology) for this study.....	10
Figure 3: Shows the fractionation process, whereby DCM, EtOAt and Ethanol solvents were pored and then eluted using vacuum pump.....	12
Figure 4: Identification of mice using permanent colored dye.....	13
Figure 5: Effect of crude extracts and fraction on blood glucose level for OGTT	20
Figure 6: Effects of ethylacetate fraction on blood glucose level of alloxan induced diabetic mice.....	23
Figure 7: Mean body weight of alloxan induced diabetic mice	24

LIST OF ABBREVIATIONS AND SYMBOLS

CMC	Carboxymethylcellulose
DCM	Dichloromethane
DM	Diabetes mellitus
EtOAc	Ethyl acetate
EtOH	Ethanol
FBG	Fasting Blood Glucose
OGTT	Oral Glucose Tolerance Test
T2D	Type II diabetes
VLC	Vacuum Liquid Chromatograph

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Diabetes mellitus (DM) is a metabolic disorder of compound etiology, delineated by prolonged hyperglycemia that is concomitant with absolute or relative inadequacy in insulin secretion or function (Srinivasan, 2017). It is the third and fastest growing disease in the world, subsequent to cardiovascular and oncological disorders according to World Health Organization (Srinivasan, 2017). Based on global estimate on diabetes prevalence, there were 371 million people with diabetes worldwide in 2012, and it is projected that the number will rise to 552 million people by 2030 (Ogurtsova *et al.*, 2015). Furthermore, Tanzania National Survey in 2012 projected an increase in diabetic population from 3.6% to 9.1% of the total adult population from 1980 to 2014 (Chacha, 2017).

According to American Diabetes Association (Ada, 2014), the overwhelming majority of diabetes cases falls under two broad etiopathogenetic categories namely (Atun *et al.*, 2017). Type 1 diabetes (insulin-dependent diabetes or juvenile-onset diabetes) caused by deficiency of insulin secretion resulting from a cellular-mediated autoimmune destruction of pancreatic β -cells. Type II diabetes (non-insulin-dependent or adult-onset diabetes) is characterized by insulin resistance which then causes insulin deficiency (Alberti & Zimmet, 1998) and accounts for 80-90% diabetes cases (Stevens *et al.*, 2007). The risk factors for type II diabetes (T2D) are unhealthy diet, tobacco use, excessive alcohol intake, overweight, obesity and physical inactivity (West & Lecture, 2011). Oral consumption of plant-based diets as well as the use of chemical and biochemical agents are currently used to control hyperglycemia (Weickert & Pfeiffer, 2008).

Besides urbanization and economic intensification, many countries have experienced dietary changes that favor increased intake of high calorific diet and decline in overall dietary quality (Hu, 2011). Unhealthy diet that is high in sugar, animal fats, fewer in fruits and vegetables has been thought to be a contributor to type II diabetes development for an extended time (Ley *et al.*, 2014). Before therapeutic use of hypoglycemic agent, diet is the most kind of treatment for the diabetes type II (Mcmacken & Shah, 2017). However, many common parts of the diet are typically urged for usual consumption, and few

taken as infusions, decoctions or alcoholic extracts (Ebers, 2019; Farzaneh & Carvalho, 2015).

Current dietary interventions in management of diabetes focus on oral consumption of plant based diet that reduces the rate of intestinal glucose absorption (glycemic index) due to high fiber content (Weickert *et al.*, 2008). Dietary fiber lowers post-prandial hyperglycemia and may additionally confer beneficial lipid-lowering and anti-hypertensive effects (Steemburgo *et al.*, 2013). Recently, the use of functional foods and their bioactive elements are thought to be a replacement approach within the hindrance and management of diabetes and its complications (Bahadoran *et al.*, 2013). Furthermore, some epidemiological investigations show that plants rich in high content phytochemicals, high total antioxidant capacity and polyphenolic compounds may perhaps be related to lowering risk of diabetes and predisposing factors, as depicted in Fig. 1 below:

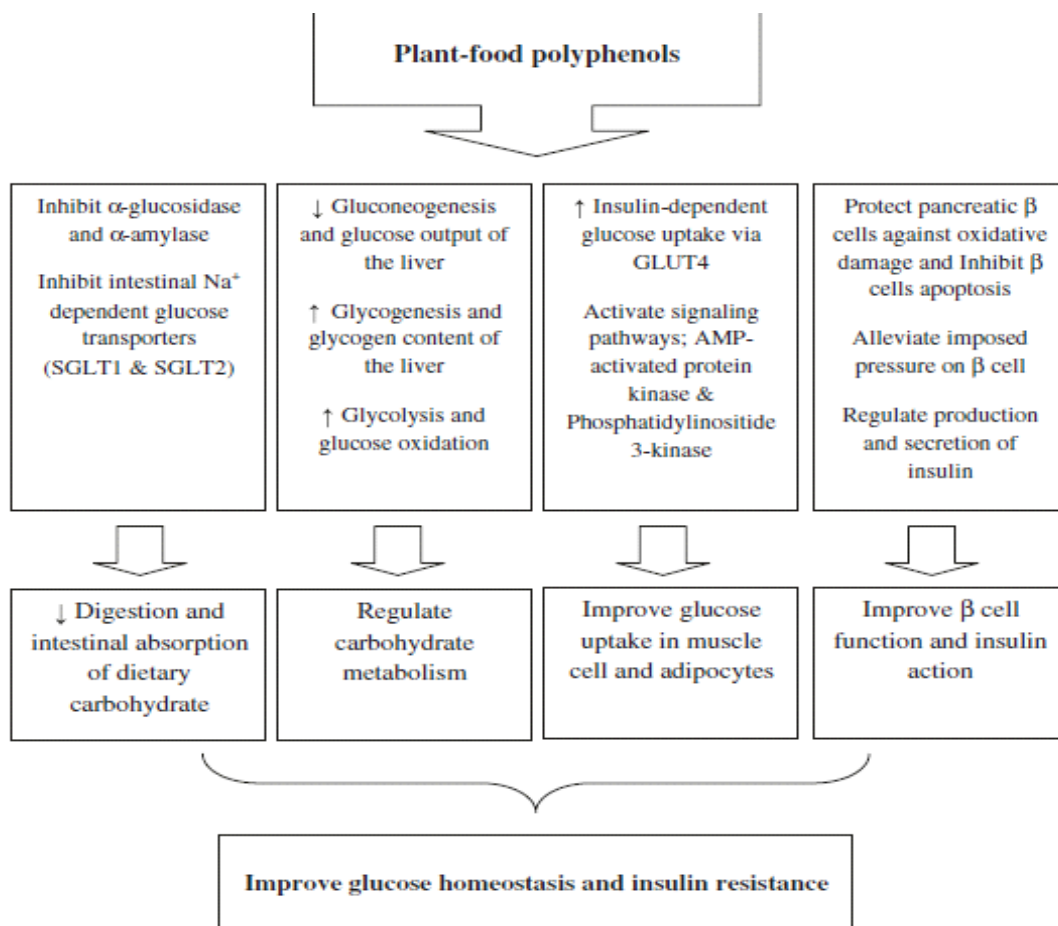


Figure 1: Schematic mechanism for function of plant polyphenols (Bahadoran *et al.*, 2013)

Cymbopogon citratus is a herbal plant within the Poaceae family commonly called lemon grass (Alberti & Zimmet, 1998). Lemon grass is utilized traditionally in Tanzania for reducing medical conditions like hyperlipidemia, hypercholesteremia and hyperglycaemia, which might lead to metabolic disorders like obesity and diabetes (Alberti & Zimmet, 1998). The anti-hyperglycaemic effects of *C. citratus* are due to high amount of alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids phytochemicals; each of which may have protective and therapeutic effect in controlling hyperglycaemia (Alberti & Zimmet, 1998). In line with Ademuyiwa *et al.* (2014) oral dosing of crude ethanolic and water extract at a dose of 200 mg/Kilogram body weight in 30 days cause significance reduction in blood glucose in albino rats (Alberti & Zimmet, 1998). However, other previous studies reported daily oral dosing of 125–500 mg/Kilogram/body weight/of fresh leaf aqueous extract of *C. citratus* in 28-42 days reduced hyperglycemia (Alberti & Zimmet, 1998).

1.2 Statement of the problem

It is estimated that 422 million people have diabetes and the prevalence has raised from 4.7% to 8.5% in 2014 worldwide (Kinimi *et al.*, 2017). In sub-Saharan African, prevalence increased from 4.7% to 7.1% from 1980 to 2014. In Tanzania the prevalence has increased to 9.1% (Chacha, 2017). The Number of people with diabetes has been observed to increase rapidly worldwide, despite current interventions (Alberti & Zimmet, 1998). Oral consumption of plant based diet as well as the use of chemical and biochemical agents are currently used to control hyperglycemia (Weickert *et al.*, 2008).

Ethno medical information has shown that consumption of *C. citratus* leaves may be used in controlling diabetes in humans (Ranade & Thiagarajan, 2015). Many of the previous studies has reported the hypoglycemic activity of crude ethanol extract (Ademuyiwa *et al.*, 2014), effects of fresh leaf aqueous extracts (Adewale *et al.*, 2007) and the effects of hot leaf extract lemon grass tea in management of diabetes (Garba, 2020). However, fractionation of the crude fraction so as to obtain the active fraction in this plant is not been documented in these studies. Also scientific studies regarding its use, bioactive compounds in fractions, and safety have not well been documented. Moreover, the efficacy of *C. citratus* formulation in treating diabetes is not well known. Therefore, this calls for scientific studies to validate the active fractions and phytochemicals claimed to be present in *C. citratus* plants, which may then be used to develop hypoglycemic drugs. This study therefore focused on evaluating the efficacy, phytochemical screening and safety of *C. citratus* fractionated extracts in controlling

hyperglycemia in diabetic mice. The aim was to affirm its hypoglycemic effect and establish baseline bioactive compounds from *C. citratus* for drug development and effective ways of using the studied plant.

1.3 Rationale of the study

Lemon grass is utilized traditionally in Tanzania for reducing medical conditions like hyperlipidemia, hypercholesteremia and hyperglycaemia, which might lead to metabolic disorders like obesity and diabetes (Alberti & Zimmet, 1998). However, fractionation of the crude fraction to obtain the active fraction in this has not been documented. Also scientific studies regarding its use, bioactive compounds in fractions and safety have not well been documented. Moreover, the efficacy of *C. citratus* formulation in treating diabetes is not well known. This study focuses on elucidate the effects of actively fractionated fraction from *C. citratus* crude extract in lowering blood glucose.

1.4 Research objectives

1.4.1 General objective

The main objective of this study was to evaluate hypoglycemic effects of *C. citratus* in alloxan-induced diabetic mice.

1.4.2 Specific objectives

- (i) To isolate crude extract and active fractions present in *C. citratus* leaves.
- (ii) To assess the influence of active fractions from *C. citratus* in controlling hyperglycemia in diabetes induced mice.
- (iii) To screen phytochemicals compounds present in actively tested fraction from *C. citratus* leaves extracts.
- (iv) To examine cytotoxicity level of active fractionated fraction of *C. citratus* in mice.

1.5 Research questions

- (i) What are the active fractions present in *C. citratus* leaves?
- (ii) Can *C. citratus* fractionated active fractions effectively control hyperglycemia?

- (iii) What are phytochemicals compounds present in actively fractionated fraction from *C. citratus* leaves?
- (iv) What are toxicity levels of actively fractionated fractions of *C. citratus* in mice?

1.6 Significance of the study

This study has added an insight on the ethno medical information pertaining to the use of *C. citratus* extracts in controlling hyperglycemia in diabetic patients. It adds value on the present countrywide and worldwide current efforts in searching for effective ways of managing hyperglycemia. It also provides benchmark information for further drug discovery studies from the *C. citratus* plants.

1.7 Delineation of the study

Delineation of study was pre-clinical study for evaluating hypoglycemic effect of *C. citratus* leaves' extract in alloxan-induced diabetic mice. Extraction process was done at Muhimbili School of Health and Allied Sciences (MUHAS) laboratory. 90 experimental mice were obtained at MUHAS while animal experimentation for Oral Glucose Tolerance Test (OGTT), Diabetic induction, diabetic treatment and acute toxicity test were done at animal house in the school of pharmacognocny. Lastly phytochemical screening was done at MUHAS laboratory.

The limiting factors that were faced during this study included; interruption during data collection due to covid 19 outbreak, less resources in the laboratory in terms of cages and extraction equipment in relation to number of researchers. Other factors included some mice resistance to alloxan induction, mice strain variability and time and budget constraint in performing sub-acute test and human experimentation.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ethnobotany in management of human diseases

Ethnobotany is the study of inter-relationship between plants and human but not restricted to study of indigenous plants by indigenous culture (Maurice, 2002). It also involves identification, taxonomy and medicinal uses of plants. Plants offer potential and nutritional values for drug development and production of various nutritional products (Venkat, 2009; Zimdahal, 2018). Variety of plant species have been documented in management and treatment of various medical conditions like hyperglycemia, hyperlipidemia, oncology and hypertension in humans (Naceiri *et al.*, 2021). This opens the way for various researchers to evaluate efficacy, mechanism of action, secondary metabolites, dosage and safety of various folklore plants. Literature data around all over the world indicates that several medicinal plants are used in management of diabetes.

2.2 Prevalence of diabetes

The number of people with diabetes increases rapidly, hence becoming a global health concern. Universally, it is predictable that 422 million people have diabetes and prevalence have raised from 4.7% to 8.5% in 2014 (Kinimi *et al.*, 2017). However, diabetes cases were anticipated to grow to 642 million by 2040 worldwide. Possible causes may include but not limited to inactivity life style, high caloric sugar intake, high fat food intake, smoking as well as alcohol intake (Zimmet *et al.*, 2016).

According to Lancet Diabetes & Endocrinology Commission Diabetes reports in sub-Saharan Africa 2017, the prevalence of type II diabetes in sub-Saharan African have increased from 4.7% to 7.1% from 1980 to 2014. Moreover, WHO established the prevalence to increase from 3.7% to 7.1% from 1980 to 2014 and projected to double by 2035 (Manne-goehler *et al.*, 2016).

In Tanzania, the prevalence of diabetes has been reported by Tanzania National Survey to increase to 9.1% of the total adult population aged 25 to 64 years in 2012 (Chacha, 2017). However, the drastic increase in diabetes has also been reported by the Ministry of Health in 2016 report. Almost 60% diabetic patients in Tanzania do not know if they are diabetic due to

weakness in health seeking behavior, misdiagnosis, poor surveillance systems and limited detection health centers (Kinimi *et al.*, 2017). Type I diabetes among children has been reported to increase to above 1200 cases in 2014 (Muze & Majaliwa, 2016). Overall, diabetes prevalence is higher among female 7.2% than male 4.7% due to gestational diabetes which occur in females (Ruhembe, 2016). Nonetheless, various studies reported remarkable variation in diabetic prevalence in rural (2%), urban (5%) and >7% of people with Asian origin (Kinimi *et al.*, 2017). Since there is inadequate data on trend of diabetic prevalence in Tanzania, it is projected that the prevalence will increase as the population increases (Kinimi *et al.*, 2017).

2.3 Diabetes management interventions

Diet plays vital role in the management of hyperglycemia (Ley *et al.*, 2014). Before starting insulin therapy, food is the foremost form of diabetes management. Management encompasses the use of plants derived customary medicines (Mcmacken & Shah, 2017). Worldwide, customary medicines from various plant sources, which are not taken as a normal food but taken as infusions, decoctions, or alcoholic extracts from herbs, spices, and vegetables have been used in management of chronic metabolic disorders like diabetes (Srinivasan, 2017). This therefore calls for intensive studies on different plant species used in prevention and management of metabolic disorders, including diabetes.

2.4 *Cymbopogon citratus* and diabetes

The *C. citratus* also known as lemon grass, is a fragrant perennial tall grass with rhizomes, a *Pinaceae* family that belongs to the genus *Cymbopogon* (Nambiar & Matela, 2012). It is widely cultivated shrub, which has originated from Malaysia, Indochina and Sri-Lanka (Adewale & Oluwatoyin, 2007). Customary lemongrass in different countries including Tanzania is used as common tea, medicinal supplement, insect repellent, insecticide, in flu control, anti-inflammatory, and analgesic (Ajayi *et al.*, 2016; Nambiar & Matela, 2012). It is potentially used in controlling medical conditions like hyperlipidemia, hypercholesteremia and hyperglycemia, which in turn may lead to metabolic disorders like obesity and diabetes mellitus (Ranade & Thiagarajan, 2015). Studies have shown that lemon grass contains alkaloids, saponins, tannins, anthraquinones, steroids, phenols and flavonoids. These phytochemicals are known for various protective and therapeutic effects in the body (Avoseh *et al.*, 2015). *Cymbopogon citratus* has been used as conventional medicine due to its

pharmacological potential from phytochemicals as an anti-diabetes effects (Uraku *et al.*, 2015).



Figure 1: Appearance of *Citrates* Nazi village in Korogwe district

Anti-diabetic activity of crude leaves extracts has been reported by different studies. Aqueous crude extract has been reported to induce anti-diabetes at 500 mg/kg of body weight within 43 days of plant treatments in Wister rats (Adewale & Oluwatoyin, 2007). However, orally administered combined lemon grass and wheat grass extract at 500 mg/kg, had also shown anti-diabetic activity (Gupta & Ak, 2017). Crude extracts from *C. citratus* contain different plants' secondary metabolites which might be responsible for anti-diabetic activity. Thus, the study evaluated the efficacy of fractionated *C. citratus* extracts on hyperglycemic mice.

2.5 Safety and toxicity of medicinal plants

Traditional herb products are generally regarded as safe to human health. This trust is the reason why traditional medicines are not accompanied by strictly prescribed doses to patients but approximation measurements. However, some medicinal plants have been reported to have toxic compounds which have side effects to human and other animals (Dzoyem *et al.*, 2014). There is therefore, a need to investigate the safety of traditional medicines to communicate to the public for precaution measure.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

This was a pre-clinical trial in which 90 albino mice were used as animal model for diabetes. Mice are the most commonly used species in preclinical research for pathophysiology of metabolic diseases, identifying pathways, mechanisms, genes regulating glucose and energy homeostasis (Thierry & Vincent, 2018). The mice in this study were randomly measured in weight, fasted, and then grouped as negative control, positive control and treatment group. The flow of activities in this study is depicted in Fig. 2 below:

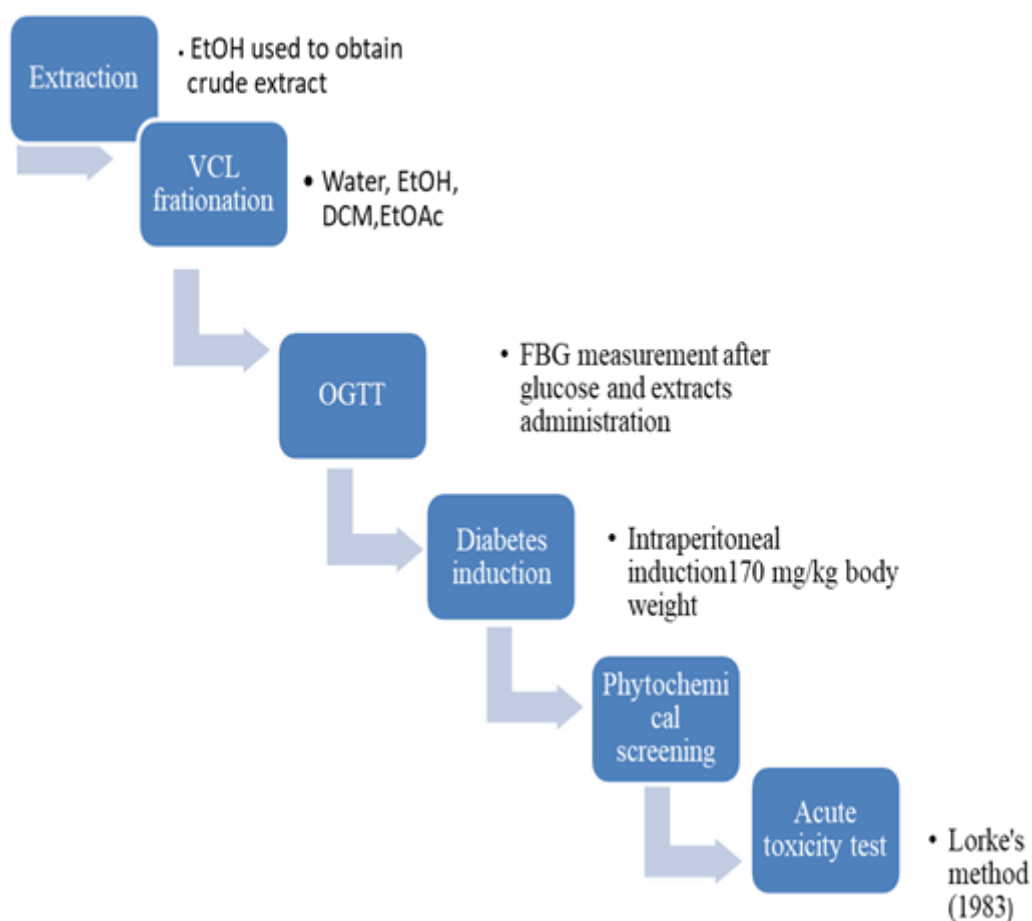


Figure 2: Flow of activities (methodology) for this study

3.2 Materials, Chemicals and Reagents

Cymbopogon citratus leaves were collected from cultivated farms at Nazi village in Korogwe District, Tanga Region, in the North Eastern highlands of Tanzania by the permission of farmers since cultivation of most spiced and medicinal plants are done. The plant was identified with voucher Not/01 by Emmanuel Mboya (Field Botanist/ Herbarium Technician) at Tropical Pesticides Research Institute (TPRI), Arusha-Tanzania. The plant leaves were shade dried for three weeks and then grinded into fine powder. The materials and equipment used in this study included: glucometer and testing strips (Glycols Inc., Canada), 1 ml syringes, gloves, masks, aluminum foil, normal saline, cotton wools, percolator, distilled water, Carboxymethylcellulose (CMC), 1% Tween 80, Methylated spirits, Ethyl acetate, Dichloromethane, Chlorpropamide tablets (Dibonis®, Cosmos Ltd, Nairobi, Kenya), 95% Ethanol (Tanzania Oxygen Ltd), Sodium pentobarbitone and Hydrochloric Acid (MERCK KILOGRAMaA group, Darmstadt, Germany).

3.3 Extraction and Fractionation of *Cymbopogon citratus*

Cymbopogon citratus powder (1700 g weight) was dissolved in 7000 ml of 95% ethanol in a percolator for 24 hours and thereafter filtered using cotton wool followed by Wolfman filter paper No. 1. 50 °C temperature was used to dry the extracts in a rotary evaporator to reduce phytochemical decomposition then allowed to air dry for 36 hours. Fractionation was performed by using Vacuum Liquid Chromatograph (VLC), whereby 30 g of unextracted ethanol extract was adsorbed with 40 g of silica gel and then crammed in a column. Solvents were poured from Dichloromethane (DCM), Ethyl Acetate (EtOA) and Ethanol (EtOH), then eluted by using a vacuum pump as shown in the Fig. 3 below:



Figure 3: Shows the fractionation process, whereby DCM, EtOAc and Ethanol solvents were poured and then eluted using vacuum pump

3.4 Extraction of *Cymbopogon citratus* water extract

Cymbopogon citratus powder weigh 100 g was poured in 1000 ml of boiled water, mixed well and then cooled. The filtrate was filtered with cotton wool and then Watman paper No.1, followed by freeze drying to obtain dry sample (Garba *et al.*, 2020). Hot water extraction by boiling resulted into decomposition of heat sensitive secondary metabolites. However, cold water extraction resulted in reduction in amount of phytochemicals (Garba *et al.*, 2020).

Percentage yield of the fractions and crude extract of *C. citratus* from 30 g of crude ethanolic extracts were obtained using the following formulas:

$$\% \text{ yield of crude extract} = \frac{\text{mass of crude extract (g)}}{\text{mass of plant materials (g)}} \times 100$$

$$\% \text{ yield of fraction} = \frac{\text{mass of the fraction (g)}}{\text{mass of crude extract (g)}} \times 100$$

3.5 Animal Selection and Identification

Albino mice were obtained from Muhimbili School of Health and Allied Science (MUHAS) at Pharmacognosy Department. Male and female albino mice of 8 to 12 weeks old weighing 20-32 grams were selected in this study. The mice were fed with food pellets and water and then exposed to light for 12 hours a day and 12 hours of darkness at night. Acclimatization was done for 4 days since the mice were found at MUHAS, which was their native environment and they had, only been moved from one cage to another within the same laboratory (Jennifer & Ransom, 2006). Prior to experiment, the mice were randomly allocated as experimental and control groups, with five (5) mice in each group for OGTT and six (6) mice for diabetic induced diabetic mice.

Identification of mice was performed using permanent colored dye for each animal in different body parts for each dosage depending on volume that will be given to mice. Pink, Yellow, blue, violet, orange and green colors were used to differentiate mice per extract as shown in Fig. 4 below:



Figure 4: Identification of mice using permanent colored dye

3.6 Oral Glucose Tolerance Test (OGTT)

Oral Glucose Tolerance Test (OGTT) was performed to obtain active fraction to be used in hyperglycemic test. Forty (40) identified mice were fasted from food and allowed access to water for 24 hours. The mice were then grouped in eight (8) groups with five (5) mice each. The material used and test procedure: Glucoplus, Glucose strip, CMC, 98% Ethanol, 1% Tween 80, Distilled water, Chlorpropamide and five (5) *C. citratus* plant extracts (crude EtOH, crude H₂O, EtOH fraction, EtOAc fraction and DCM fraction).

3.6.1 Experimental procedure

The experimental mice were grouped in eight (8) groups with five (5) mice each. Group 1 and 2 were negative control groups, which included CMC and 1% Tween-80, which were administered respectively. CMC was used as the mobile solvent for dissolving crude H₂O extract, crude EtOH and EtOH fraction extract where by 1% Tween-80 was used as the mobile solvent for dissolving DCM and EtOAc fractions hence used as negative control in respect to their dissolved fractions.

Group 3 and 4 mice were administered with water and ethanol crude extract at 400 mg/kilogram body weight dose. Crude water extracts were used because traditionally people use hot water extract in management of diabetes, hence the need to evaluate if there it has significance hypoglycemic activity in comparison to crude ethanol extract used in the study. Group 5, 6 and 7: DCM, EtOAc and EtOH fractions were orally administered by using gavage in mice respectively, at the constant ratio dose of 400mg/kilogram body weight right after glucose load (Uzor *et al.*, 2014). Group 8 mice were orally administered with chlorpropamide as the standard drug after glucose load since treatment compliance was reported to be higher in patients using it in management of diabetes (Andrew *et.al* 1985). Dose used was 400 mg/kilogram body weight.

Prior to experiment, Fasting Blood Glucose (FBG) for each mouse was measured and recorded as t=0. The mice were administered with CMC, 1% Tween 80, crude H₂O, crude EtOH, DCM fraction, EtOAc fraction and EtOH fraction. A read-made oral glucose loading dose of 1g/kilogram body weight was given by gavage to each mouse 30 minutes later. Hyperglycemic level for each mouse at each dose was then taken at 0.5, 1, 2- and 3-hours intervals after glucose loading dose by using glucometer (Andrea *et al.*, 2001, Thierry & Poitout, 2017 & Raquel *et al.*, 2020).

3.7 Induction of diabetes in mice

Before diabetic induction, 30 mice were fasted for 24 hours, their weight and FBG were measured and recorded as t=0. The mice were injected with a single intraperitoneal administration of freshly prepared alloxan monohydrate in normal saline at 170 mg/kilogram body weight dose. The mice were then provided with mice feed pellets 30 minutes later.

Diabetic symptoms such as increased rate of urination (polyuria), water intake due to thirsty (polydipsia), high rate of food consumption (polyphagia) and body weakness were observed monitored in the experimental mice induced with alloxan within 72 hours (Szkudelski, 2001). FBG test was performed after six hours where the mice with FBG greater/ equal to 11.1 mmol/L were considered diabetic (Sofiano *et al.*, 2018). Twenty (20) mice turned to be diabetic, five mice died during intraperitoneal dosage administration and the remaining five mice were resistant to alloxan induction.

3.8 Extract administration and hypoglycemic activity in mice

Hypoglycemic activities of *C. citratus* EtOAc fraction were tested against diabetic induced mice. Diabetic mice were orally administered with ethyl acetate fraction 72 hours after alloxan induction at 400 mg/kilogram body weight daily. The study was performed for 20 days whereby FBG and weight were measured on days 1, 5, 10, 15 and 20 of treatment. Treatments were carried out using the following materials *C. citratus* EtOAc fraction Gavage, Chlorpropamide and 1% Tween 80.

3.8.1 Experimental test procedures

Diabetic induction resulted into 20 diabetic mice after 170 mg/kg body weight alloxan induction within 72 hours monitoring (Lenzen, 2008). The mice were grouped into three (3) groups where by group one (1) contains 8 mice then were administered with 1% Tween 80. Group two (2) contained six (6) mice administered with EtOAc fraction which was the active fraction during OGTT. Group three (3) contained six (6) mice administered with chlorpropamide as positive control at 400 mg/kilogram body weight dosage.

3.9 Test animal handling

The use of mice followed internationally accepted principles for European Union (EU) directive 2010/63/EU on the use of animals for research (Szkudelski, 2001). After the study,

the experimented mice were euthanized by intraperitoneal dosing of 200 mg/Kilogram body weight of 18% Sodium pentobarbitone (Bryony, 1996) in accordance with AVMA guideline for euthanasia of animals 2020 edition (CPCSEA, 2003), followed then by their disposal at Muhimbili National Hospital incinerator.

3.10 Phytochemical screening of ethyl acetate fraction

Qualitative tests for determining secondary metabolites present in the ethyl acetate fraction were performed using the following materials: *C. citraus* EtOAc fraction, Wagner's reagent, 10% Lead acetate solution, 1% Lead acetate solution, Chloroform, Concentrated sulphuric acid, Benedict's solution and 5% sodium bicarbonate solution.

3.10.1 Experimental procedures

The following tests were conducted in this phytochemical screening part:

- (i) Test for alkaloids: Wagner's test: 10 mg of extract was taken, and then few drops of Wagner's reagent were added. The formation of a reddish-brown precipitate indicated the presence of alkaloids.
- (ii) Test for Flavonoids: Lead acetate test: 10 mg of extract was taken and few drops of 10% lead acetate solution were added. The appearance of the yellow color precipitate indicated the presence of flavonoids.
- (iii) Test for Phenols and Tannins: Lead acetate test: 10 mg of extract was taken and 0.5 ml of 1% lead acetate solution was added. The formation of precipitate indicated the presence of tannins and phenolic compounds.
- (iv) Test for steroids and sterols: Salkowski's test: 5 mg of extract was dissolved in 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turned red and the lower layer turned yellow with green fluorescence, indicating the presence of steroids and sterol compound in the extract.
- (v) Test for Carbohydrates: Benedict's test: 5 mls of Benedict's solution was added to 0.5 mg of extract and boiled in a water bath. The appearance of red or yellow or green precipitates indicated the presence of reducing sugars.

- (vi) Test for Saponins: Honey comb test: 0.5 mg of extract was taken in a test tube and few drops of 5% sodium bicarbonate solution were added. The mixture was shaken vigorously and kept for 3 minutes. The formation of honey comb-like froth indicated the presence of saponins.

3.11 Acute toxicity test

Lorke's (1983) was used to test plant toxicity, in which eighteen (18) mice were grouped into three groups and then administered with different doses (10, 100 and 1000 mg/kilogram) of ethyl acetate fraction and crude water extracts per group. The mice were then kept under observation for 24 hours to observe their behavior and mortality then followed five days after treatment. No mortality in phase one was observed in mice. Six (6) mice were grouped in three (3) groups of one (1) mouse each in phase two. The mice were administered with ethyl acetate fraction and crude water extract at 1600, 2900 and 5000 mg/kilogram body weight doses (Nchinedu *et al.*, 2013). The mice were then placed under observation for 5 days to observe their behavior and mortality.

3.12 Hypoglycemic statistical data analysis

Results were expressed as mean \pm SD. Comparison among groups was analyzed using One-way ANOVA. The difference between the means of the two population groups against negative control was considered significant at $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Extracts and fractions yields

The total yield of extraction was 2.35%. The fractions and their percentage yield are represented in Table 1. The fraction percentage yield of plant ranges from 0.09 (2.7 g) to 0.27 (7.7 g). Dichloromethane fraction yield highest fraction followed by ethanol fraction than EA fraction. The results showed that there is variability in yield due to amount and nature of phytochemicals extracted in each fraction.

Table 1: Fractions and their percentage yield

Fractions	Mass extracted	% yield
Ethanol crude	40g	2.35
Ethanol fraction	5.4g	0.18
DCM fraction	7.7g	0.27
EA fraction	2.7g	0.09

4.2 Oral glucose tolerance test (OGTT)

The mean blood glucose for experimental mice varied with time. After glucose loading dose at 0.5 hour, the overall mean glucose level increased for some tested groups except for ethyl acetate fractioned standard drug. All extracts showed subsequent decrease in mean blood glucose load with respect to their negative control after 1 hour of glucose load. During the 2nd and 3rd hour after glucose load the mean blood glucose for all groups dropped at fairly constant rate as shown in Table 2. Moreover, ethyl acetate fraction showed significant hypoglycemic activity when compared to negative control (1% T80).

Table 2: Mean FBG (mmol/l) for OGTT

Time (hrs)	Mean blood glucose level (mmol/L) \pm S. D n=5							
	Controls			Crude Extracts and fractions at 400 mg/kgBW				
	CMC	1%T80	Positive	H ₂ O crude	Ethanol crude	Ethanol fraction	DCM fraction	EA fraction
0	3.16 \pm 0.86	7.24 \pm 2.45	5.82 \pm 0.40	2.96 \pm 0.57	2.9 \pm 0.59	3.4 \pm 0.80	7.14 \pm 1.80	8.44 \pm 1.43
0.5	3.52 \pm 1.03	9.50 \pm 2.11	3.22 \pm 0.35	5.1 \pm 1.35	4.66 \pm 1.46	4.82 \pm 2.45	7.9 \pm 3.08	7.68 \pm 2.14
1	3.22 \pm 0.67	5.46 \pm 0.61	4.34 \pm 1.11	4.3 \pm 1.15	3.68 \pm 0.79	3.7 \pm 1.06	7.14 \pm 2.88	5.68 \pm 1.51
2	3.1 \pm 0.62	4.86 \pm 0.51	3.14 \pm 0.47	4.22 \pm 1.07	3.46 \pm 0.56	3.28 \pm 0.78	4.8 \pm 0.51	4.28 \pm 0.86
3	3.22 \pm 1.00	3.94 \pm 0.38	3.16 \pm 0.60	3.78 \pm 0.50	3.46 \pm 0.60	3.16 \pm 0.91	4.02 \pm 0.50	3.62 \pm 0.69

Ethyl acetate and DCM fractions showed overall statistically significant reduction in blood glucose level ($p = 0.004$ and $= 0.007$, at 95% confidence level) when compared to negative control (1% T80). Both crude extracts and fractions showed different responses on glucose lowering as shown in Fig. 5 below:

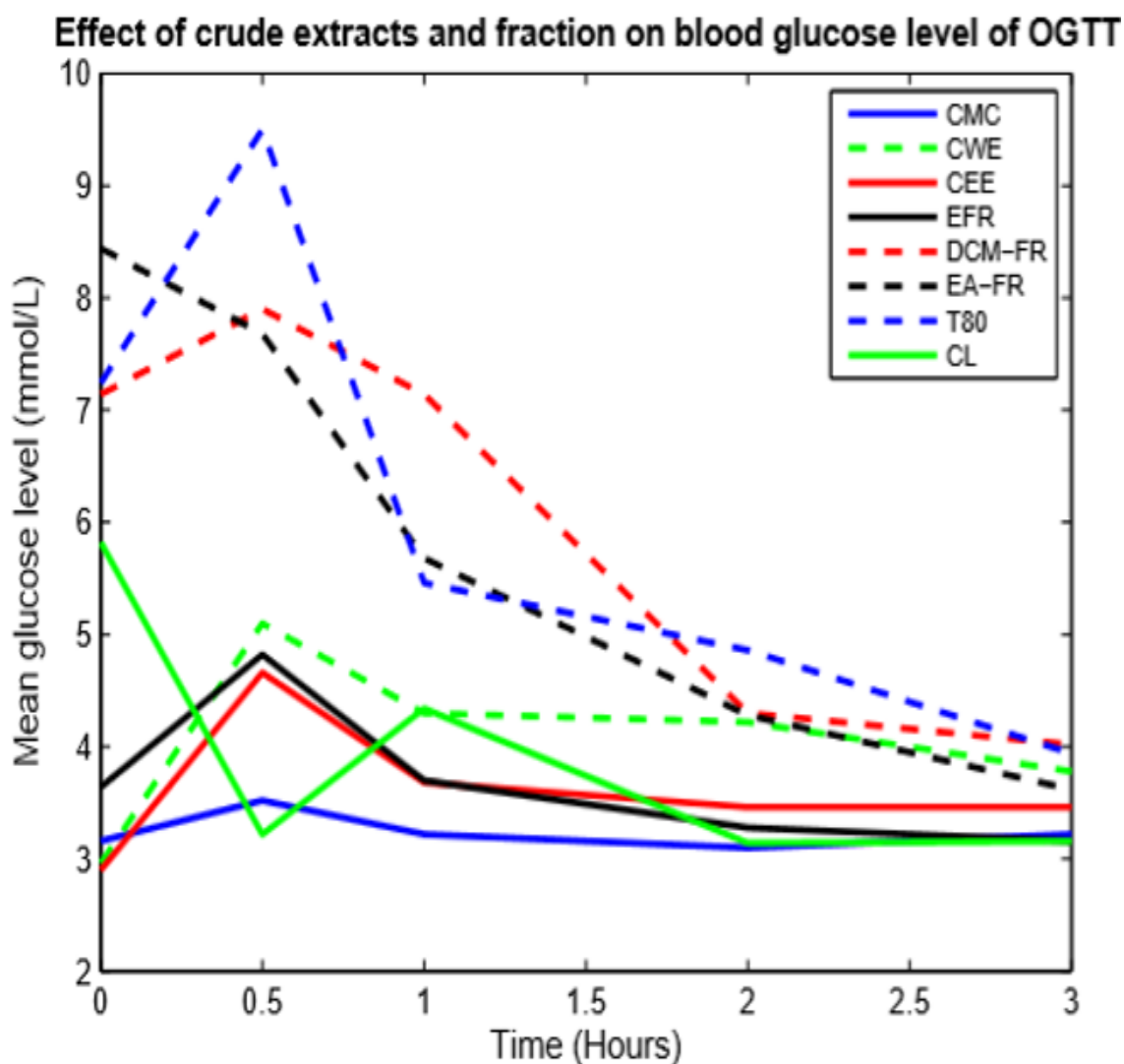


Figure 5: Effect of crude extracts and fraction on blood glucose level for OGTT

NB: CMC- Carboxymethylcellulose, CWE- Crude Water Extract, CEE-Crude Ethanol Extract, EFR- Ethanol fraction, DCM- FR-Dichloromethane fraction, EA-FR- Ethylacetate fraction, T80- Tween80 and CL- chlorpropamide

Almost 1200 species of indigenous herb plants are traditionally used in treatment and management of diabetes worldwide (Farzaneh & Carvalho, 2015). *Cymbopogon citratus* plant have hypoglycemic effects in normal and diabetic mice (Boaduo *et al.*, 2014; Lunyera *et al.*, 2016; Garba *et al.*, 2020). Hypoglycemic activity of lemon grass is due to interaction of various bioactive chemical compounds (secondary metabolites) or several compounds in

isolation (Zhou *et al.*, 2016). Oral glucose loading is a physiological induction of diabetes mellitus by fleetingly increasing the blood glucose level of experimental animal without pancreas damage for diagnosis of impaired glucose tolerance, diabetes mellitus, and gestational diabetes (Singh & Pathak, 2015). The results in Table 2 showed that, administration of both crude extracts and fractions from *C. citratus* at 400 mg/kg BW resulted into steady decrease in blood glucose level within 90 minutes of treatment. Moreover, the findings showed that, ethyl acetate fraction had significant ($p = 0.004$) reduction of blood glucose where hyperglycemic activity increased as polarity extraction decreased. Variation in hypoglycemic activity of *C. citratus* crude extracts and fractions may be due to presence of bioactive compounds, antagonistic effects or synergetic effects, which may occur between compounds present in extracts or fractions (Ademuyiwa *et al.*, 2014; Gupta & Ak, 2017; Garba *et al.*, 2020). Furthermore, dosage variations for different medicinal plants have been reported to influence hypoglycemia. For example, administration of 250 and 500 mg/kg body weight of ethanolic *Psidium guajava* decreased blood glucose levels in oral glucose tolerance test model in rats (Mazumdar *et al.*, 2015) whereas 200 mg/kg body weight of aqueous *Moringa oleifera* extracts significantly reduced blood glucose in normal and diabetic rats (Jaiswal *et al.*, 2013).

4.3 Diabetes induction test

Diabetes induction test was performed following modification of study done by Szkudelski in 2001. Day 0 was the day before alloxan induction where FBG was measured, followed by 178 mg/kg BW alloxan induction. Elevation of FBG was observed between 48-72 hrs after alloxan induction approximately three to four times of normal mice. Increased rate of urination (polyuria), water intake due to thirsty (polydipsia), high rate of food consumption (polyphagia) and body weakness were observed in the experimental mice induced with alloxan.

The mean FBG level for 20 days of treatment is presented in Table 3 below. The mean glucose level for chlorpropamide (positive control) and ethyl acetate fraction decreased from day 5 contrary to 1% Teen 80 (negative control) in which FBG increased. Decreased mean glucose level was observed in days 10, 15 and 20 for positive control and ethyl acetate fraction. However, mean glucose level increased for negative control as shown in Fig. 4. Moreover, mean body weight decreased in diabetic mice during the experiment as shown in Table 3 below. Unremitting decrease in mean body weight was also observed for ethyl

acetate fraction from day 1 to day 20 but increased body weight was observed in the positive control group in first 5 days and was associated with irregular decrease in mean body weight for the experimental mice as shown in Fig. 6.

Table 3: Mean FBG (mmol/l) for alloxan induce diabetes mice

Time (Days)	Mean blood glucose level (mmol/L) \pm S.D n=5					
	Controls				Fraction	
	Positive		1%T80		Ethyl acetate	
0	5.52	\pm 0.82	5.82	\pm 0.35	5.72 \pm 0.70	
1	16.06	\pm 5.87	13.35	\pm 3.29	24.8 \pm 9.43	
5	10.8	\pm 6.08	15.03	\pm 5.65	14.86 \pm 10.47	
10	8.1	\pm 3.70	17.55	\pm 6.90	11.72 \pm 6.39	
15	6.24	\pm 1.67	16.45	\pm 5.16	10.25 \pm 5.10	
20	4.65	\pm 1.02	18.45	\pm 5.92	8.725 \pm 1.83	

Effects of ethylacetate fraction on blood glucose level of alloxan induced diabetic mice

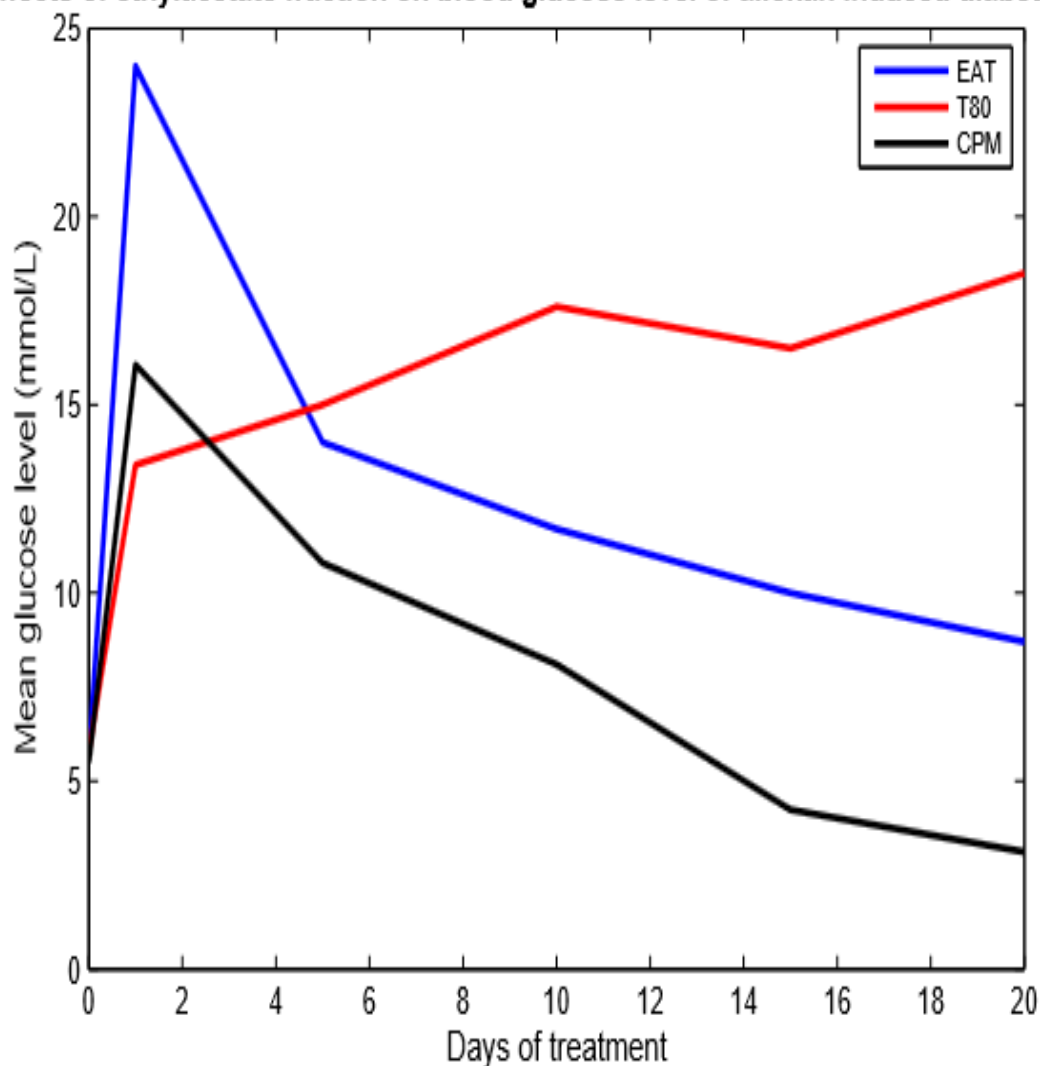


Figure 6: Effects of ethylacetate fraction on blood glucose level of alloxan induced diabetic mice

NB: EA-FR- Ethylacetate fraction, T80- Tween80 and CPM- chlorpropamide.

Table 4: Mean body weights of alloxan treated mice

Time (days)	Mean body weights \pm S.D (g), n = 5		
	Control		Fraction
	Positive	1%T80	Ethyl acetate
0	25.2 \pm 5.45	25.67 \pm 1.03	28 \pm 3.94
1	25.4 \pm 5.32	25.33 \pm 1.63	28 \pm 3.94
5	27 \pm 6.04	26.17 \pm 2.56	26.2 \pm 3.56
10	26.4 \pm 7.16	25.67 \pm 1.75	24.8 \pm 3.11
15	23.8 \pm 5.98	24 \pm 2.50	23.25 \pm 2.83
20	21.4 \pm 3.22	23.83 \pm 2.48	22 \pm 2.83

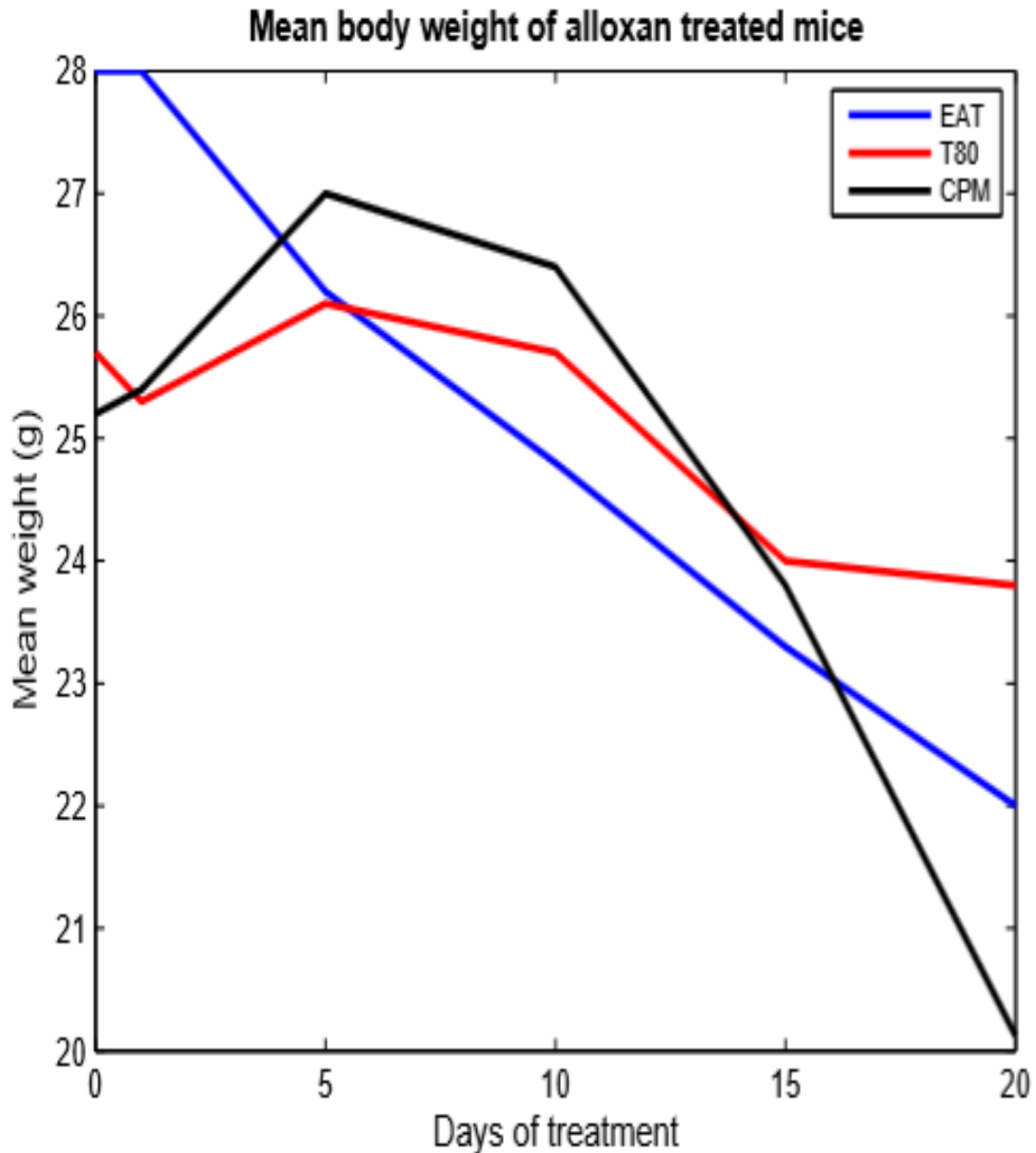


Figure 7: Mean body weight of alloxan induced diabetic mice

Alloxan induces diabetes by selectively inhibiting glucose-induced insulin secretion through specific inhibition of glucokinase (glucose sensor for beta cells). It causes a state of insulin-dependent diabetes through its ability to induce ROS formation resulting in selective necrosis of beta cells (Lenzen, 2008). It is known that alloxan has diabetic action when administered intravenously, intraperitoneally or subcutaneously. However, dosage administration depends on animal species, route of administration and nutritional status of an animal. This study showed that, administration of 170 mg/kg BW of alloxan was sufficient to induce diabetes in albino mice used in the study. The intraperitoneal dose below 150 mg/kg BW may be insufficient for inducing diabetes in mice and rat (Szkudelski, 2001). The mean

FBG for 20 days of ethyl acetate fraction treatment showed statistically significant reduction in blood glucose as indicated in Table 3 above. Increased blood glucose was observed in albino mice treated with 1% T80, since it was used as negative control for hypoglycemic action comparison. Decreased blood glucose was also associated with steady decrease in body weight for the treated mice and positive control group due to decrease on plasma cholesterol for mice treated with *C. citratus* as seen in Fig. 7 (Adeneye, 2007). However, continuous increase in weight was observed in mice treated with 1% T80 which might be contributed by high rate of water and food while remaining untreated. Observed hypoglycemic activity of *C. citratus* from ethyl acetate fraction may be contributed by bioactive compounds present in the fraction (Gomes *et al.*, 2009; Piero *et al.*, 2011).

4.4 Phytochemical screening of *Cymbopogon citratus* ethyl acetate fraction

Preliminary phytochemical screening of ethylacetate fraction showed presence of alkaloids, phenol and tannin, saponin and anthraquinone as shown in table 5 below.

Table 5: Phytochemical screening of *Cymbopogon citratus* ethylacetate fraction

Plant phytochemical	Ethyl acetate fraction	Test name
Alkaloids	+	Wagner's test
Flavonoids	-	Lead Acetate test
Phenol and Tannin	+	Lead Acetate test
Steroids	-	Salkowski's test
Carbohydrates	-	Benedicts test
Saponins	+	Honey Comb test
Glycosides	-	Glycosides test
Protein	-	Biuret test
Anthraquinone	+	Bontrager's test

NB: + indicates present of tested phytochemical and - indicates absent of tested phytochemical

Several mechanisms might contribute to the observed hypoglycemic effects from *C. citratus* ethyl acetate extract including inhibition of glucose absorption in the intestine, stimulation of insulin secretion from pancreatic β -cells, modulation of glucose release from the liver activation of insulin receptors and glucose uptake in insulin-sensitive tissues, modulation of intracellular signaling pathways and gene expression. The mentioned conditions may be contributed by alkaloids, saponin, antraquinone, phenols and tannins present in the *C. citratus* extract/fraction (Adewale & Oluwatoyin, 2007; Keun *et al.*, 2005). Two anthraquinones

namely: chrysophanol-8-*O*- β -d-glucopyranoside and chrysophanol were isolated from *Korean rhubarb* rhizome and found to enhance glucose transport in myotubes with moderate cytotoxicity (Myung & Cheon, 2008). The chrysophanol-8-*O*- β -d-glucopyranoside compound was found to significantly enhance insulin-stimulated glucose transport by insulin receptor activation. Moreover, Chrysophanol influenced insulin responsive glucose transport by increasing GLUT4 mRNA expression (Ota & Ulrih, 2017). Another anthraquinone, Sennidin A, was found to induce the translocation of GLUT4 in rat adipocytes (Abe *et al.*, 2006). On the other hand, saponins are naturally occurring surface active glycosides mainly produced by plants. It helps to reduce cholesterol levels, by improve lipid metabolism and may help prevent and treat obesity of which are common in type 2 diabetes (Ota & Ulrih, 2017). The actions of the two secondary metabolites (saponins and anthraquinones) found in *C. citratus* ethyl acetate fraction in this study may also have been responsible for the observed hypoglycemic activity in the treated albino mice in this study. Further studies on these compounds are therefore warranted which involved isolation of individual bioactive compound to observe their hypoglycemic effects.

4.5 Acute toxicity test

No mortality was observed in mice from all the two experimental phases in phase one and two on the acute toxicity tests. However, some behavioral changes were observed in mice in the first hour of the acute toxicity experiment as shown in Table 6. Tremors and excessive sleep were observed in mice treated with both crude water extracts and ethyl acetate fraction at a dose above 1000 mg/kgBW.

Table 6: Mice behavioral changes

Behaviors	Phase 1 (mg/kgBW)						Phase 2 (mg/kgBW)					
	Water crude			Ethyl acetate fraction			Water crude			Ethyl acetate fraction		
	10	100	1000	10	100	1000	1600	2900	5000	1600	2900	5000
Tremors	X	X	✓	X	X	✓	✓	✓	✓	✓	✓	✓
Convulsions	X	X	X	X	X	X	X	X	X	X	X	X
Salvation	X	X	X	X	X	X	X	X	X	X	X	X
Diarrhea	X	X	X	X	X	X	X	X	X	X	X	X
Lethargy	X	X	X	X	X	X	X	X	X	X	X	X
Sleep	X	X	✓	X	X	✓	✓	✓	✓	✓	✓	✓
Respiratory changes	X	X	X	X	X	X	X	X	X	X	X	X
Mortality	X	X	X	X	X	X	X	X	X	X	X	X

NB: X - indicates absence of indicated behavior at specific dose

✓ - indicates presence of indicated behavior at specific dose

Although most herbal products are regarded as safe without strictly prescribed doses, some medicinal plants are reported to have side effects in both human and other animals (Dzoyem *et al.*, 2014). The toxicity results from this study showed that *C. citratus* water extract and ethyl acetate fraction was non-toxic since no mortality was observed at a dose up to 5000 mg/kg BW following 5 days after single oral plants extracts administration. However, mild toxicological signs were observed in the form of active movement of the mice in 10 minutes of extract administration, followed by tremors and deep sleep at the dose above 1000 mg/kg BW. These results are in accordance with findings from study by Adewale and Oluwatoyin (2007), where hepatotoxic and nephrotoxic effects were also reported in mice treated with fluid extracts from *C. citratus*. These findings suggest that *C. citratus* use should not be taken at very high doses (above 1000 mg/kg BW) when used as hypoglycemic agent to ensure personal safety.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Considering significant hypoglycemic activities in mice and body weight reduction by ethyl acetate fraction demonstrated in this study, *C. citratus* extracts can possibly be used in controlling hyperglycemia.

The findings from this study support the use of *C. Citratus* leaves in controlling hyperglycemia. However, precautions should be taken on the dosage frequency and time of use, since mild toxicological signs were observed during acute toxicity test including active movement of the mice in 10 minutes of extract administration, followed by tremors and deep sleeping at the dose above 1000 mg/kg BW. However, this may not be the case with humans who have already been using this herb for many disease conditions as reviewed in this study.

5.2 Recommendations

Further study on active compounds (possibly anthraquinones and saponins) from studied *C. citratus* extract is therefore warranted.

Finally, detailed clinical trial should also be done, including prior sub-acute toxicity test to verify their safety in organs.

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RESEARCH OUTPUTS

Publication Paper

Temu, J., Martin., H. D & Saul, E. (2020). Hypoglycemic Effect of *Cymbopogon Citratus* Leaves' Extract and it's Fractions in Alloxan-Induced Diabetic Mice. *International Journal of Tropical Diseases and Health*, 41(22), 1-11.

Poster Presentation

Hypoglycemic Effect of *Cymbopogon Citratus* Leaves' Extract and it's Fractions in Alloxan-Induced Diabetic Mice.